

Isamu UMEZAKI\*: **The germination of tetraspores of  
*Hildenbrandia prototypus* Nardo and its life history**

梅崎 勇\*: 紅藻植物エンジマダラの四分胞子の  
発生と生活史に関する研究

(Plates V~VIII)

Ogata (1954), studying the germination of tetraspores of *Hildenbrandia* sp. from Izu, central Japan, believed that it was very doubtful that the genus *Hildenbrandia* belonged to the Corallinaceae or the Squamariaceae because of their germination pattern, and therefore to be regarded as a member of the Hildenbrandiaceae. Flint (1955), who published his study on freshwater species, *H. rivularis*, from the United States, said that the first cell divisions which took place in the germination of the tetraspores were in a plane parallel to the axis. The mode of germination in this freshwater species, however, is different from that of a marine species, *H. prototypus* investigated by Chemin (1937) and *Hild.* sp. by Ogata (l.c.). Although sexual reproduction and especially female reproductive structures are essential characters for the classification of the Florideophycidae, the size of spore and the type of germination of spores may be also used as additional characters (Inoh 1947). Ogata's study was only on the earliest stages in germination and gave few details on the further development.

Gemmae, stolons, fragmentation and monospores are known not only as vegetative and asexual reproduction but also as important parts in the life history of *H. rivularis* (Starmach 1952, Flint 1955, Nichols 1965, Seto & Hirose 1968). For the present, no full information is known on the life history of the marine species, *H. prototypus*. On the other hand, only a few ecological observations were made by Rosenvinge (1917) and Printz (1926).

Since December 1965 the writer has been engaged in systematic and life-historical studies of *Hildenbrandia prototypus* by means of cultural experiments of the tetraspores.

\* Department of Fisheries, Faculty of Agriculture, Kyoto University, Nagahama, Maizuru, Japan.  
舞鶴市長浜京都大学農学部水産学教室.

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**Materials and methods** *Hildenbrandia prototypus* Nardo grows on granite rock walls in the littoral zone (between effective high water mark and low water mark), in patches about 40 cm wide, particularly at the upper levels. The species is perennial. The red-brown, encrusting plant is firmly adherent to rocks, growing during all seasons of the year, although in winter (February and March) the upper level plants slightly decay and spore liberation is interrupted. The encrusting plant was collected together with the rock substratum and were broken into small pieces and washed carefully with the sterilized sea water to remove diatoms and other minute contaminants. One or two small pieces of the materials were put in a watch-glass and spore were later liberated. Tetraspores were discharged one or two hours later or at latest within 24 hours. The discharged spores were transferred by a micropipette to Petri dishes on the bottom of which slide glasses were placed. After germination the slide glasses to which early germlings attached were again transferred to culture vessels in which Provasoli's enriched seawater medium, SWII solution, was added for culture. The medium was suitable for the growth of the germlings of tetraspores. Culture vessels were placed near windows whose glasses were pasted with thin tracing papers in order to avoid direct rays of the sun and cultured at room temperature. The lowest temperature in the laboratory room was 2 to 5°C in winter and the highest in summer, 30°C. The culture solution were changed every one or two weeks.

In order to study changes in the liberation of tetraspores the encrusted plants were collected once or twice a month from the same locality during the whole course of this study, and spores made liberate.

The work has been carried out at the Department of Fisheries, Faculty of Agriculture, Kyoto University, which is located on the coast of Nagahama, Maizuru Bay from which materials were collected, during the period between December 1965 and June 1968.

### Observations

In general, most of the tetraspores were discharged as a mass of four spores united together just as they were within the sporangial wall in the

conceptacle. (Text-fig. 1 and plate V, fig. 1). The top of the tetrasporic masses was thick and rounded and the other end at which they were probably attached to the inside wall of conceptacle was slightly attenuate. Most of the discharged spore masses had dividing walls which run obliquely or irregularly between spores, but in some septa were obscure. The length of spore masses varies between  $35\ \mu$  and  $48\ \mu$ , the breadth between  $10\ \mu$  and  $15\ \mu$ . Soon after discharge dividing walls cut off the tetraspore mass into four spores,

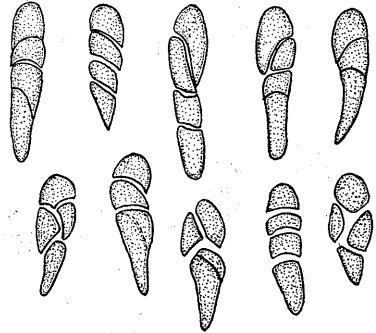


Fig. 1. Showing various types of tetrasporic masses in the shape of which they were discharged.  $\times 500$ .

each being a tetraspore. For a short time after that the four spores showed amoeboid movement in order to separate from each other. Usually, a few hours later, spores fastened to the slide glass and became spherical. The spore contained many small pale red granules of floridean starch and was invested with a membrane. It has a single large parietal chromatophore within which no pyrenoid was observed. The fastened spores were  $10\text{--}17\ \mu$  in diameter, mostly  $11\text{--}12\ \mu$ . (Text-fig. 2a, b and plate V, fig. 2). In most cases they began to germinate immediately after fastening to the substratum or at latest within 24 hours. Generally, in the warmer seasons spores began to germinate very soon after fastening to the substratum, while in the colder seasons it was very slow to germinate.

The spherical spore first protruded at its one pole and elongated itself the same length as the spore body or two or three times longer. (Text-fig. 2 and plate V, fig. 3). With the elongation of the germ tube the protoplasm of the spore body migrated into it, the entire contents being transferred usually within 24 hours. A small quantity of protoplasm occasionally remained in the spore body, although it looked emptied. Then, a wall formed to divide the emptied spore body from the germ tube, which later developed into a disc thallus. Placed under weak illumination such as 500 lux and at low temperature such as  $5^{\circ}$  to  $10^{\circ}\text{C}$ , the germling spore did not produce the first dividing wall, although in later stages walls run irregularly. Under these conditions, they continued to grow abnormally, as shown in text-figures 3 and

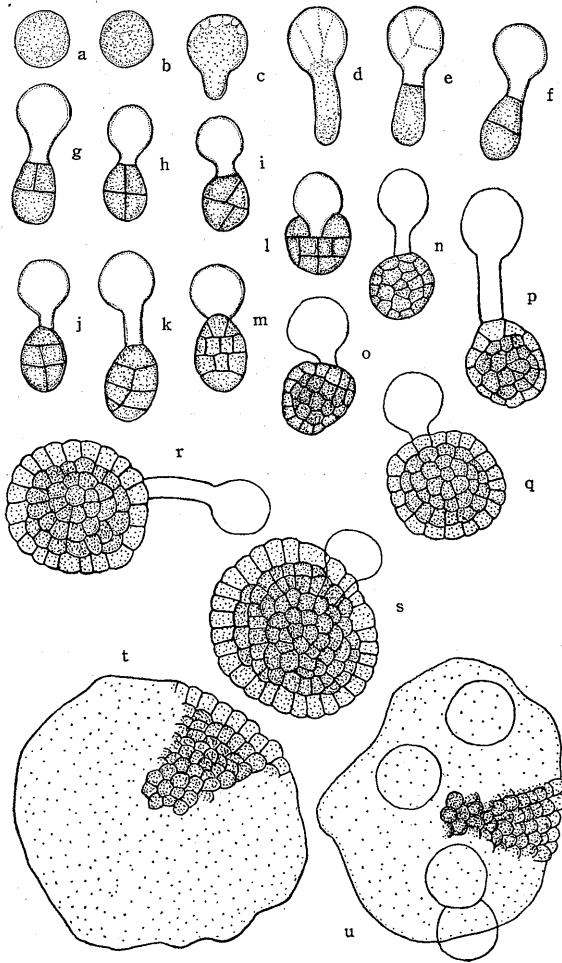


Fig. 2. a, b. Tetraspores. c, d. Germination of spores. e. Formation of the first walls cut off between spore body and germ tube. f. Formation of the second wall which runs parallel to the first wall. g. Formation of the third wall. h, i. Formation of the third and fourth walls simultaneously. j-n. Successive cell divisions by which monostromatic circular discs are formed. o, p. Stages in the formation of distromatic discs. q-t. Discs in which central parts are composed of three or four stromatic layers. u. Discs thallus in which four germlings were overlapped each other. a-u.  $\times 400$ .

4. However, when the abnormals were placed under normal conditions, they began to divide and developed into normal discs. The normal germling, on the other hand, which had been under normal conditions from starting, formed its second dividing plane parallel to the first wall, then becoming two cells. This appears to be a special feature for the germination of tetraspores of *Hildenbrandia*, although the character does not agree with that of *H. rivularis* investigated by Flint (l.c.). Soon after the one or two cells so formed became a little larger in size, the third and fourth divisions whose planes run perpendicularly or obliquely to the second wall took place successively or simultaneously, forming three or four cells. Afterwards, each of the germlings divided successively and

increased cells on its marginal portion. After one month in culture the germlings developed into circular monostromatic disc consisting of twenty to thirty cells. At this stage the central part of the disc began to divide by planes parallel to the substratum to form two layers of cells. (Plate VI, figs. 1-3). Among early cultures some germlings divided by planes parallel to the first wall to form one row of cells, composed usually of three to eight

cells. Afterwards, they produced discs at the tops of their filaments by means of normal process. (Text-fig. 3 m, n and 4 f-i). The discs, which became 25-40  $\mu$  in size, were all distromatic at their center. The cells of the disc were quadrate or pentagonal in surface view and were 2.5-3  $\mu \times$  3.5-4  $\mu$  in size. In older discs their peripheries became irregular in outline, probably due to the irregular growth of the marginal cells, which sometimes ramified in dichotomous manner. When the disc thalli became about 20  $\mu$  thick and 1 mm broad, their central parts were tetrastromatic because of repeated periclinal cell divisions. (Plate VI, fig. 6). The vertical cells of the thallus are isodiametric except basal ones, and are quadrate, 3.5-3.7  $\mu$  wide, 3.5-3.7  $\mu$  high, the dimensions being similar to those of collections from the sea. The basal cells are a little longer and larger as compared with the upper ones and some of them were sometimes decayed. As the plant continued to grow the cells increased towards the tips by forming horizontal walls. Polystromatic regions increased in extent radially, but the margins remained monostromatic. At a stage when

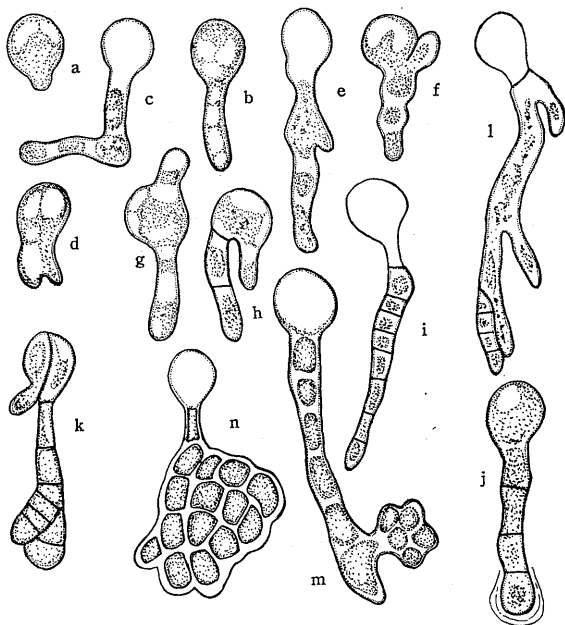


Fig. 3. Abnormal germlings showing various stages in development.  $\times 400$ .

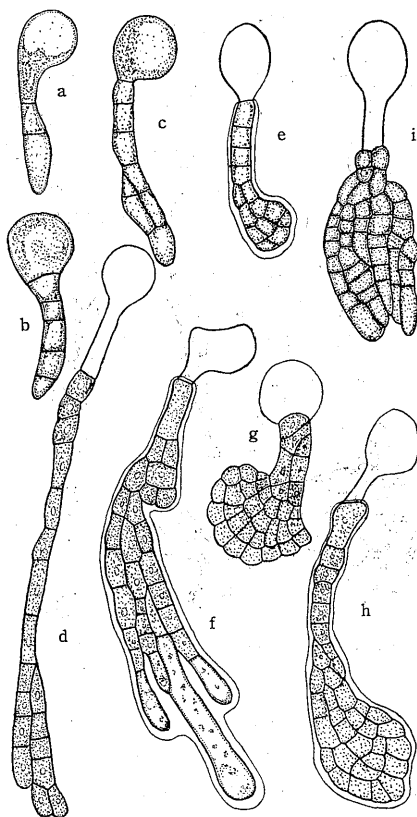


Fig. 4. Various stages of abnormal germlings in development and a process of disc formation from their germlings.  $\times 400$ .

ness of 70–100  $\mu$ . The chromatophore of the thallus cells is parietal and often lobed at the margins and single or 2–3 a cell. At that stage each germling overlapped each other, forming encrusting thalli whose general appearance resembles those in the sea. (Plate VIII, fig. 1). Disc thalli, which germinated in December 1965 when this study started had been maintained and by June 1968 had reached 5–13 mm in diameter, although nearly all of them were overlapped. (Plate VIII, fig. 2). Even in such well-developed thalli neither conceptacles nor other reproductive organs were formed on them.

On a crust plant whose lower surface was detached from the slide-glass,

the discs became 1.5 mm broad and 25  $\mu$  thick, their vertical filaments were about ten cells in number, but their marginal parts were still monostromatic. When the discs were 5 mm in diameter, their vertical sections were composed of 15–20 layers of cells and about 70  $\mu$  thick. Even in such old thalli the monostromatic condition was still retained near the margins and their marginal extensions continued. (Plate VII). The cells of the margin were pale purple or nearly colorless, large-elongate, usually peculiar in shape as compared with other inner cells and measured 3.5–4  $\mu$  wide, 5–15  $\mu$  long. (Text-fig. 5 and plate VIII, fig. 3). After approximately eight months the cultured plants grew up into ones which were morphologically indistinguishable from plants from nature and became a diameter of 5–10 mm and a thick-

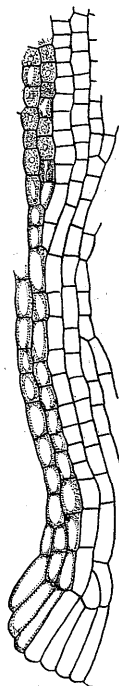


Fig. 5. Part of the monostromatic periphery of a large disc thallus showing an arrangement of cells and chromatophores in surface view.  $\times 400$ .

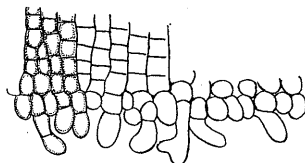


Fig. 6. Part of a thallus in vertical section showing rhizoid-like cells sent out from its base.  $\times 400$ .

rhizoid-like cells composed of one or two cells were found. (Text-fig. 6 and plate VIII, fig. 4). But, it does not seem that they correspond to those of a freshwater species of *H. rivularis* (Fritsch 1929, Geitler 1932, Yoneda 1949, Seto & Hirose 1968), because they were very short as compared with the latter.

Since December 1965 when the present culture work began the crust plants of the species were collected from the same locality once or twice a month to observe changes of discharging of tetraspores. Tetraspores were discharged throughout the year except for the months of February and March. Late in January the number was rapidly decreased and in the middle of February

discharge was stopped. And late in March they were again discharged. The some observations were made in the years 1966, 1967, and 1968. During the winter season plants growing near high water mark decayed and because of this changed themselves into yellow-brown color, or sometimes completely disappeared. On the other hand, crust plants which grew near low tide level, were still alive, although no spore liberation was found. Through the course of this investigation no any other reproductive cells and organs such as monospores, spermatangia and carposporophytes were observed on the materials from Maizuru Bay.

### Discussion

In 1868, the family Hildenbrandiaceae was established by Rabenhorst. The family was used by Schmitz (Hauck, Meeresalgen, 1882), although later abandoned by himself (1889), who considered *Hildenbrandia* as a systematically uncertain genus. In 1897, Schmitz & Hauptfleisch placed it among the Corallinaceae as a doubtful genus. De Toni (1905) placed it with the Squamariaceae in a subfam. Hildenbrandtieae. On the other hand, Rosenvinge (1917) thought the genus represented a separate family Hildenbrandiaceae intermediate between the Squamariaceae and the Corallinaceae. According to recent classification the genus is placed either under the Hildenbrandiaceae (Papenfuss 1955, Kylin 1956, Fott 1959, Lund 1959, Humm 1962, Gayral 1966) or under the Squamariaceae (Taylor 1957, 1960, Chapman 1963, Hollenberg & Abbott 1966). Fritsch (1945) regarded it a genus of uncertain position allied to the Corallinaceae. Melchior (1954) also entertained a doubt upon its systematic position, although he placed it in the Squamariaceae. The classification mentioned above were made by characters, mainly such as the presence of lime, the presence of conceptacles of sporangia, and direction of division of sporangia.

From the point of view of the germination of tetraspores Ogata (1954) suggested that it was very doubtful to ally *Hildenbrandia* with either the Corallinaceae or Squamariaceae and it is reasonable to place it in the Hildenbrandiaceae. Moreover, he considered that the family Hildenbrandiaceae represented a group systematically lower than Corallinaceae and Squamariaceae. In the Corallinaceae (*Amphiroa dilatata*, Inoh 1947; *Jania rubens*, Thuret & Bornet 1878; *Corallina pilulifera*, Hasegawa & Kakui 1958) and the Squamariaceae (*Peyssonnelia squamaria*, Killian 1914) the germination mode of spores is immediate discal type of Inoh (1947) from which that of *Hildenbrandia* (Chemin 1937, Ogata 1954, the present study) is sharply different, although Flint (1955) reported in *H. rivularis* another type of germination. As Ogata (l.c.) following Inoh (1947) has indicated, the Hildenbrandiaceae should have a lower phylogenetic position than Corallinaceae and Squamariaceae because of smaller spore size and simpler type of germination. Although sexual plants whose carposporophytes are essential characters for the systematic classification of the Florideophycidae are unknown in *Hildenbrandia*, it

seems for the present that the genus is best placed among the Hildenbrandiaceae.

According to Rosenvinge (1917) the plants of *Hildenbrandia prototypus* grew for the whole year on the Denmark coast and also its sporangia were found produced at all times of the year, arising successively in the conceptacle, although activity diminished only in the season of winter. Printz (1926) also observed on the Trondhjemfjord in Norway that the vegetative plants were perennial, although its conceptacle and sporangial formations were seasonal. Feldmann (1951) included the species in a group of perennial algae in his ecological classification of marine algae. The work on species from Maizuru Bay at which the present work was carried out agrees with these observations. Considered from these studies the marine species appear to reproduce only by tetraspores without other reproductive methods.

On the other hand, propagation by gemmae, stolons, fragmentation, and monospores are well known as reproductive methods in the freshwater species *H. rivularis* (Starmach 1952, Flint 1955, Nichols 1965, Seto & Hirose 1968) and they are considered to play a more important role than tetraspores for the life cycle of the species. In the freshwater species the existence of rhizoids are reported (Fritsch 1929, Geitler 1932, Yoneda 1949, Seto & Hirose 1968). Recently, Seto & Hirose (l. c.) observed in *H. rivularis* that in culture the end of a rhizoid developed into a prostrate system. These processes showed resemblance to some germlings in early stages of the present cultures in which disc thalli were produced at the ends of filaments elongated from spores. (Text-fig. 4). Moreover, from the facts that the marginal parts of disc thalli continued to grow for a long time in culture, it seems that the plant continues to grow throughout the year except for the cold season in the sea. It appears that this plays an important role in vegetative propagation. Moreover, from a point of view that liberation of tetraspores were found throughout the year except for a short period in winter, it seems that the species of Maizuru Bay is reproduced chiefly by tetraspores, although it has additional vegetative propagation as mentioned above. With respect to this genus, many problems such as cytological studies of tetrasporangia and sexual reproduction remain unsolved.

### Summary

1. Germination of tetraspores of *Hildenbrandia prototypus* Nardo and its life history were studied.
2. Tetraspores were discharged as a mass of four tetraspores and were 10–17  $\mu$  in diameter, mostly 12–13  $\mu$ . The germinating mode was Inoh's mediate discal type, which agreed well with that of Chemin (1937) and Ogata (1954).
3. Considered from the germinating mode and the spore size of tetraspores the genus *Hildenbrandia* should be placed under the Hildenbrandiaceae and the family should be grouped systematically lower than Corallinaceae and Squamariaceae, until the sexual reproduction is known.
4. It was found that the species of *Hildenbrandia* from Maizuru Bay is reproduced chiefly by tetraspores. Moreover, it was regarded that the species is vegetatively propagated in the sea, from the observations that the margins of disc thalli in culture continued to grow for a long time.
5. Rhizoid-like cells, which were sent out from the lower surface of a crust plant, were observed.

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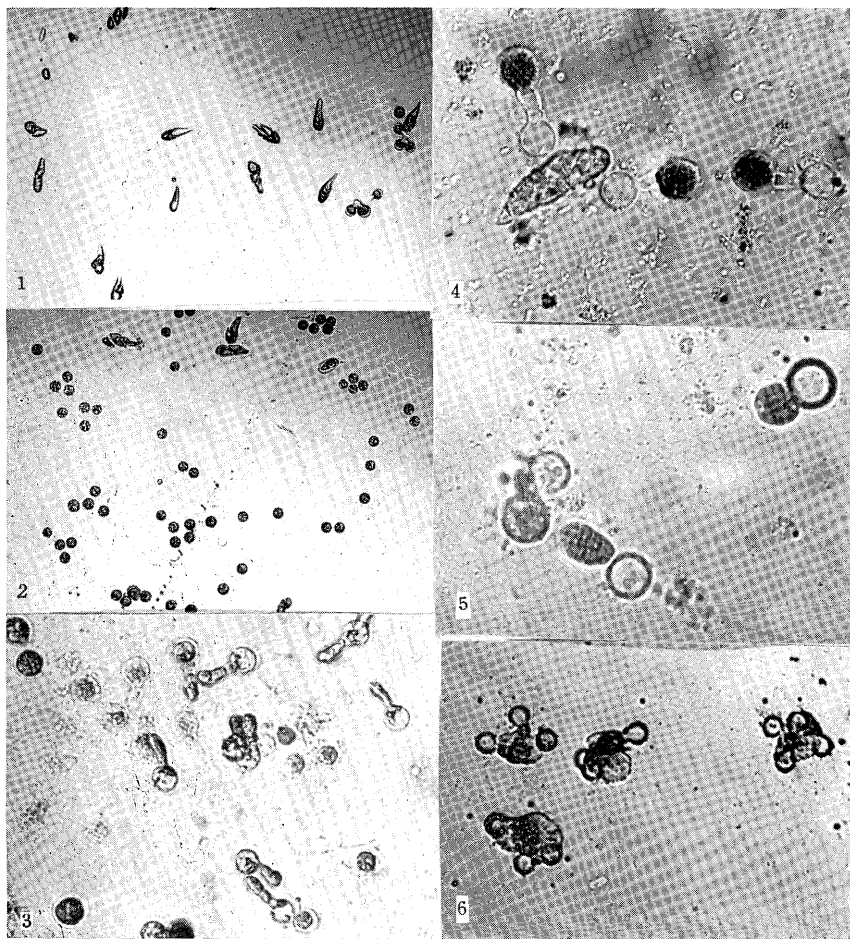
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### Explanation of plates

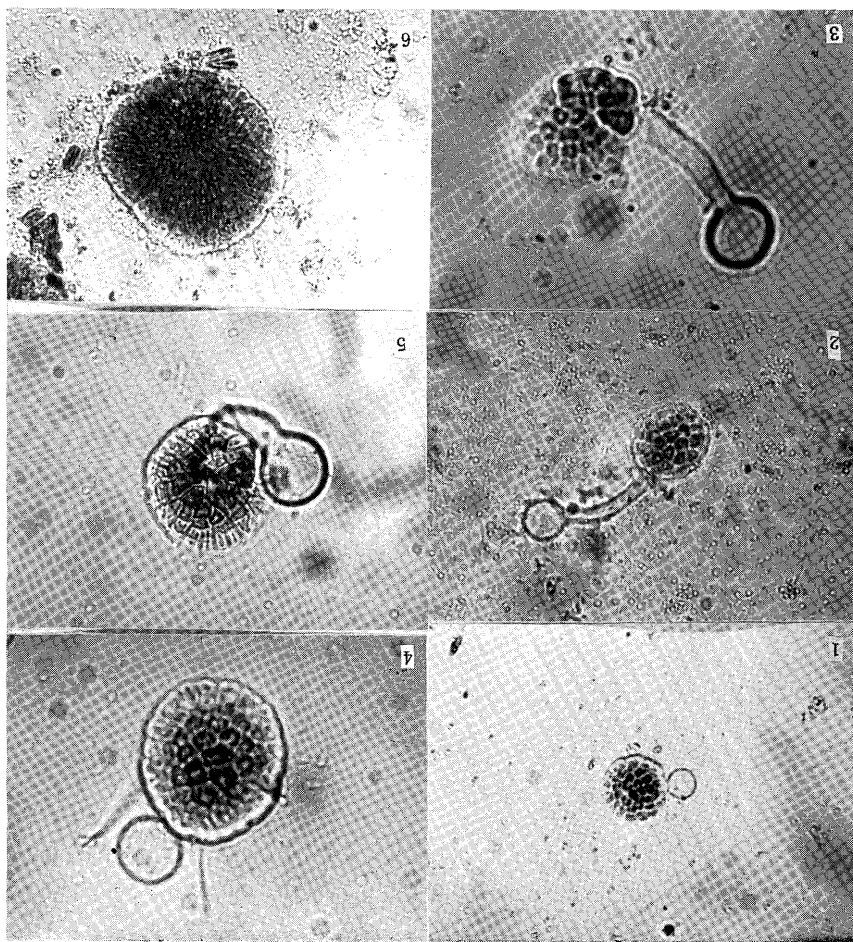
- Plate V. Fig. 1. Various types of tetrasporic masses on a slide-glass just after discharge.  $\times 100$ . Fig. 2. Tetraspores and tetrasporic masses.  $\times 100$ . Fig. 3. Beginning of the germination of spores.  $\times 250$ . Figs. 4, 5. Early stages of disc formation.  $\times 350$ . Fig. 6. Germ-lings of four adjacent tetraspores which were originated from the same sporangium.  $\times 250$ .
- Plate VI. Figs. 1-3. Thalli in distromatic stage. 1  $\times 200$ , 2  $\times 250$ , 3  $\times 400$ . Figs. 4, 5. Thalli in tristromatic stage.  $\times 400$ . Fig. 6. Thallus in tetra- or pentastromatic stage in its central part.  $\times 400$ .
- Plate VII. Various shape of disc thalli in a half-year to a year cultures. 1  $\times 25$ ; 2, 4  $\times 30$ ; 3, 6  $\times 15$ ; 5  $\times 20$ .
- Plate VIII. Fig. 1. Disc thalli growing on slide-glass in one year culture.  $\times 1$ . Fig. 2. Disc thalli growing on slide-glass in two years culture.  $\times 1$ . Fig. 3. Part of the margin of a disc thallus showing its surface view and its irregular growth in outline.  $\times 75$ . Fig. 4. Part of a disc thallus in vertical section showing rhizoid-like cells.  $\times 400$ .

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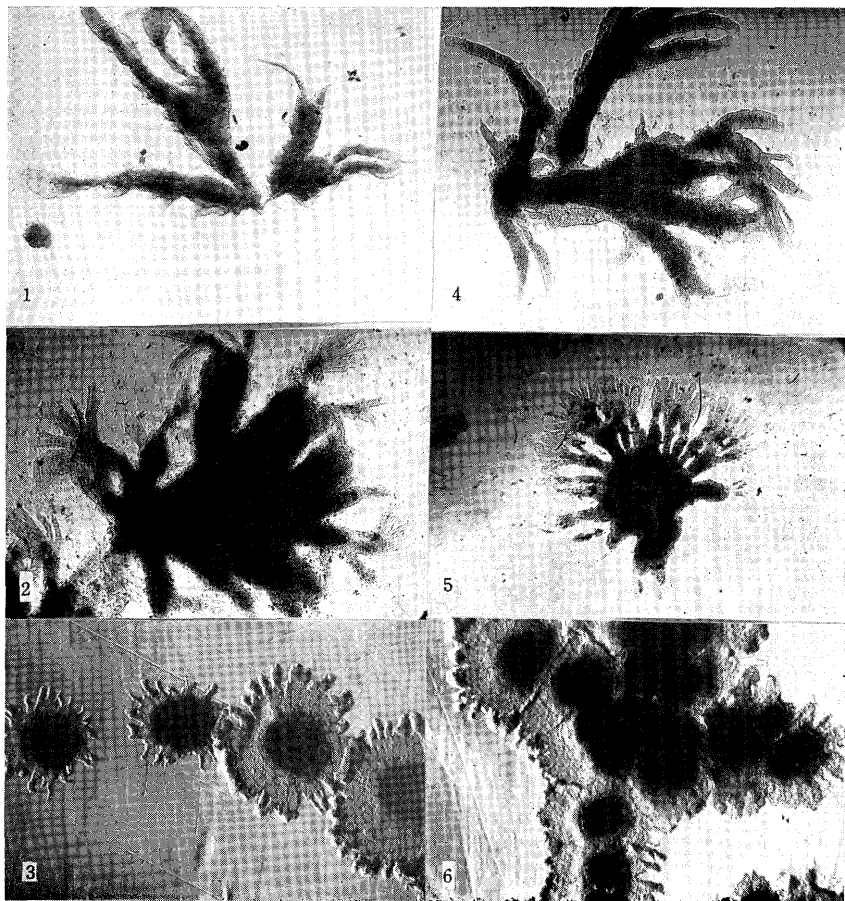
エンジマダラの四分胞子の発生様式は間接盤状型であった。その発生様式と胞子の大きさからベニマダラ属 (*Hildenbrandia*) はベニマダラ科に置くべきであり、また同科は系統学的にイワノカワ科およびサンゴモ科より下位に属するものと考えられた。発芽体は、まず一層細胞よりなる球形盤状体をつくり、ついで、その基質と平行な面で分裂が起こり多層の盤状体となる。盤状体周辺部は常に一層細胞よりなり、一年を通じて生長を続けた。この事実から、舞鶴湾産の本藻は四分胞子による他に、常に栄養生長も行っているものと考えられた。冬期の 2-3 月を除き、一年を通じて四分胞子の放出と発生がみられた。本藻は、冬期には潮間帯上部に生育するものは枯死消失するが、その下部のものは生存し、一年間その生育がみられた。培養植物体上にも、海からの採集品にも、無性芽、単胞子、精子器、および果胞子体が観察されなかった。培養した盤状体基部より仮根様細胞の発出しているのがみられた。



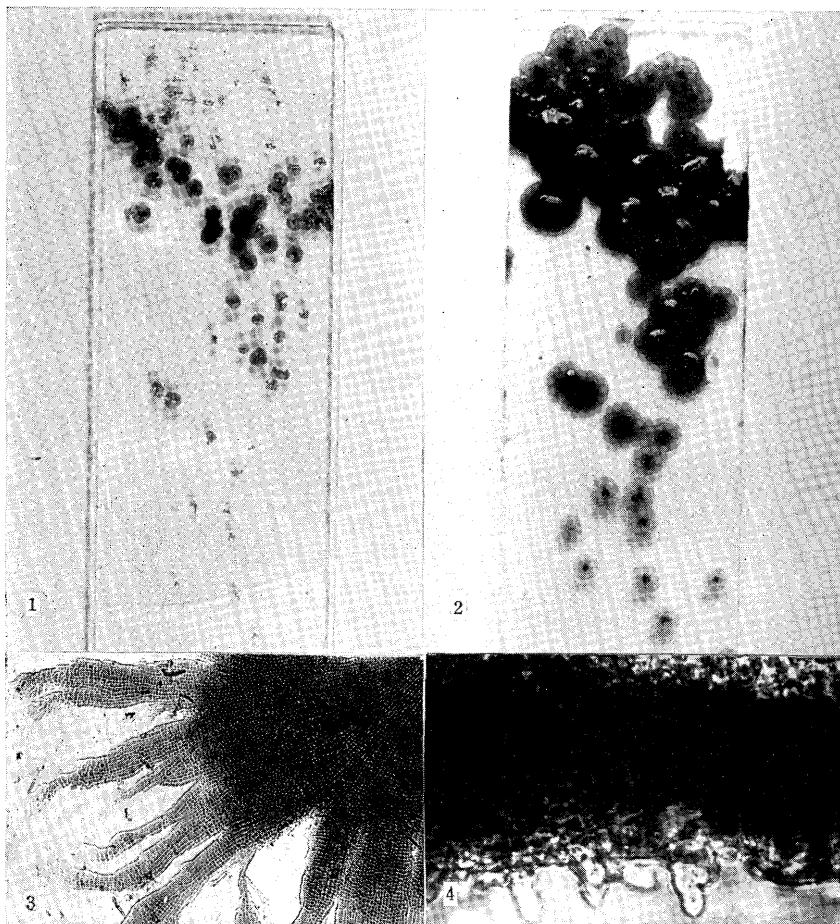
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